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Seroprevalence of Human Herpesvirus 8 in Human Immunodeficiency Virus 1-Positive and Human Immunodeficiency Virus 1-Negative Populations in Japan

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To determine the seroprevalence of human herpesvirus 8 (HHV8) among human immunodeficiency virus 1 (HIV-1)-positive (HIV-1$^+$) and HIV-1-negative (HIV-1$^-$) populations in Japan, 276 HIV-1$^+$ patients and 1,000 HIV-1$^-$ blood donors were enrolled in this study. Antibodies against HHV8 latency-associated nuclear antigen (LANA) were examined through indirect immunofluorescent assay by using a B-cell line that was infected latently with HHV8 (body cavity-based lymphoma 1). An HHV8$^-$ and Epstein-Barr virus-negative B-cell line (Ramos) was used as a control. Thirty-two seropositive cases against LANA (anti-LANA$^+$) were identified among the 276 HIV-1$^+$ patients who were studied. Five cases were foreigners living in Japan. The risk factor of all 27 Japanese cases was unprotected sexual intercourse, and the great majority of these cases (23 in 27; 85%) reported homosexual/bisexual behavior. Anti-LANA$^+$ status correlated with the presence of sexually transmitted diseases, such as amoeba and HBV infection, further suggesting male homosexual behavior as the main route of HHV8 transmission in Japan. Only two LANA$^+$ cases were identified among 1,000 HIV$^-$ blood donors in Japan; thus, seroprevalence of HHV8 identified by LANA was estimated to be 0.2% among HIV-1$^-$ populations in this country. ***J. Med. Virol. 57:159–162, 1999.***

KEY WORDS: human herpesvirus 8; Kaposi's sarcoma; seroprevalence

INTRODUCTION

Human herpesvirus 8 (HHV8), also known as Kaposi's sarcoma (KS)-related herpesvirus (KSHV), was first identified as a KS-specific DNA fragment in late 1994 [Chang et al., 1994]. Based on its high sequence homology to Epstein-Barr virus (EBV) and its capacity to grow in B-lymphocytes, HHV8 has been considered a member of the γ-herpesviruses. HHV8 is also associated with other neoplastic diseases, such as body cavity-based lymphoma (BCBL) [Cesarman et al., 1995] and multicentric Castleman's disease [Karcher and Alkan, 1995; Soulier et al., 1995]. Although precise analyses of the causative role of HHV8 in the formation of these tumors are still needed, epidemiological studies have suggested classifying HHV8 as a human tumor virus. HHV8 has been identified in not only human immunodeficiency virus (HIV)-related but also in endemic KS cases in central Africa [Lennette et al., 1996]. HHV8 has been detected in normal peripheral B lymphocytes in patients with these tumors.

Here, we report HHV8 seroprevalence among HIV-1-positive (HIV-1$^+$) and HIV-1-negative (HIV-1$^-$) populations in Japan. We also correlate the presence of HHV8 with other infectious diseases in the HIV-1$^+$ cohort. Our results contribute not only to epidemiological studies of the virus but also to the public health and blood safety discourses in Japan.

PATIENTS AND METHODS

Patients

HIV-1$^+$ patients (n = 276) were enrolled in this study: 247 males (89.5%) and 29 females (10.5%; Table I). Eighteen patients were foreigners living in Japan. Among the 258 Japanese patients, unprotected sexual

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TABLE I. Human Herpesvirus 8 Seroprevalence in Japan

Population	Risk factor	Number	Positive (%)[a]
HIV-1$^+$			
Japanese	Unprotected sexual intercourse		
	Homo/bisexual	85	23 (27.1; 95%C.I., 17.7–36.6)
	Heterosexual	54	4 (7.4)
	Hemophilia and other coagulopathies	118	0
	Transfusion	1	0
Foreigner	Unprotected sexual intercourse		
	Homo/bisexual	5	1 (20.0)
	Heterosexual	12	4 (33.3)
	Transfusion	1	0
Total		276	32 (11.6; 95%C.I., 7.8–15.4)
HIV-1$^-$			
Japanese	—	1,000	2 (0.2)

[a]C.I., confidence interval.

intercourse (USI) was a risk factor for 139 patients (53.9%), 85 patients reported homosexual/bisexual intercourse, and 54 patients reported heterosexual intercourse as a risk factor. One hundred and eighteen (45.7%) were patients with hemophilia or other blood coagulation disorders (one case each with von Willebrand disease and factor X deficiency) who were infected through contaminated blood products. One patient (0.4%) was infected through blood transfusion. Intravenous drug use is a rare risk factor for HIV-1 infection in Japan, and such cases were not present in our cohort. Among 18 foreigners, five reported homosexual/bisexual intercourse, 12 reported heterosexual intercourse, and one reported blood transfusion as risk factors. EDTA plasmas of HIV-1$^+$ patients were obtained with informed consent and stored at −80°C in the Department of Infectious Diseases, Institute of Medical Science. Samples obtained between 1995 and 1997 were used in this study. Citrated plasmas from blood donors were obtained from the Japanese Red Cross Central Blood Center and were stored at −20°C. All plasma samples were heated to 56°C for 30 minutes and were stored at −20°C until use.

Immunofluorescent Assay of Antilatency-Associated Nuclear Antigen Antibodies

BCBL-1 is an HHV8$^+$ and EBV$^-$ human B-cell line established from BCBL [Cesarman et al., 1995]. Ramos is an HHV8$^-$ and EBV$^-$ human B-cell line [Klein et al., 1975]. BCBL-1 was obtained from the IDS Research and Reference Reagent Program (National Institutes of Health, Bethesda, MD). Ramos was obtained from the American Tissue Culture Collection (ATCC, Rockville, MD). Cells were maintained at 37°C in 5% CO_2 with RPMI 1640 supplemented with 10% fetal calf serum (FCS), 0.02% $NaHCO_3$, 100 U/ml penicillin, and 100 μg/ml streptomycin. Immunofluorescent assay (IFA) was performed as described previously [Kedes et al., 1996]. Briefly, cells were washed twice with phosphate-buffered saline (PBS) and resuspended to achieve a concentration of 1×10^5 cells/ml. One hundred microliters of each cell suspension were spun down (500 rpm for 2 minutes) onto a siliconized glass slide by using Cytospin (Shandon, Pittsburgh, PA). Cells were fixed with methanol-acetone (1:1) at room temperature for 5–minutes, transferred into PBS. and stored at 4°C until use. Cells on the glass slides were preblocked and incubated with patient plasma diluted 1:40 in masterblock (MB; PBS supplemented with 1% glysine, 2% bovine serum albumin, 10% goat serum) at 37°C for 1–2 hours. Cells were washed for 1 hour in PBS with 0.4% Tween-20 and then twice in PBS (1 hour/wash). After removing excess PBS, cells were incubated for 40 minutes in a humidified chamber with secondary antibodies conjugated with Texas red diluted 1:100 in MB. The secondary antibodies used were F(ab′)2 fragments of goat anti-human immunoglobulin G (IgG) F(ab′)2 fragment (Rockland, PA). After incubation, excess secondary antibodies were aspirated, and cells were washed as described above. Slides were mounted with fluorescent mounting medium (DAKO, Carpenteria, CA) and inspected by using a fluorescent microscope (microphone-FAX; Nikon, Tokyo, Japan) at magnifications from ×600 to ×1,000. Samples were considered true antilatency-associated nuclear antigen (anti-LANA) antibody-positive (anti-LANA$^+$) when all BCBL-1 nuclei produced a discrete, speckled pattern, whereas all Ramos nuclei remained unstained. Immunofluorescent analysis was performed blind by a researcher (T. Fujii) who possessed no information about the samples.

Serological Status Against Various Pathogens

Serological studies on various pathogens were performed by using the following standard methods: syphilis, *Treponema pallidum* hemagglutination test (TPHA); amoeba, IFA on anti-amoeba antibodies; toxoplasma, passive hemagglutination test (PHA) or enzyme-linked immunosorbent assay (EIA) on anti-toxoplasma IgG; hepatitis B virus surface antigen (HBsAg), reversed passive hemagglutination (RPHA) or EIA; anti-hepatitis B virus surface antibodies (HBsAb), PHA or EIA; hepatitis C virus (HCV), particle agglutination (PA) on anti-HCV antibodies; human T-cell leukemia virus type I (HTLV-I), PA on anti-HTLV-I anti-

TABLE II. Incidences of Various Infectious Diseases Among HIV-1$^+$ Individuals According to the Risk Factors of HIV-1 Infection (Group I or II) and Latency-Associated Nuclear Antigen Status (Group Ia or Ib)†

					P value*	
	Group I (%)	Group Ia (%)	Group Ib (%)	Group II (%)	Ia vs. Ib	I vs. II
Risk factor	All USI	LANA$^+$ USI	LANA$^-$ USI	CBP		
Total number	156	32	122	120		
Age (mean ± S.D.)	37.5 ± 10.4	43.8 ± 9.6	35.3 ± 8.7	29.3 ± 8.7		
Syphilis (TPHA)	52/122 (42.6)	17/30 (56.7)	35/92 (38.0)	0/51 (0)	>0.05	$<1.0 \times 10^{-12}$
Amoeba	19/89 (21.3)	9/24 (37.5)	10/65 (15.4)	ND	<0.05	ND
Toxoplasma	16/112 (14.3)	8/30 (26.7)	8/82 (9.8)	4/75 (5.3)	<0.05	<0.05
HBV (HBsAg)	15/138 (10.9)	3/30 (10.0)	12/108 (11.1)	6/102 (5.9)	>0.26	>0.1
HBV (HBsAb)	56/131 (42.7)	18/27 (66.7)	38/104 (36.5)	67/99 (67.7)	<0.005	<0.0001
HCV	8/136 (5.9)	4/32 (12.5)	4/104 (3.9)	104/107 (97.2)	>0.07	$<1.0 \times 10^{-53}$
HTLV-I	3/81 (3.7)	1/14 (7.1)	2/67 (3.0)	2/62 (3.2)	>0.36	>0.34
HSV-I	71/82 (86.6)	19/19 (100)	52/63 (82.5)	27/66 (40.9)	<0.05	$<1.0 \times 10^{-8}$
VZV	76/83 (91.6)	20/21 (95.2)	56/62 (90.3)	57/65 (87.7)	>0.31	>0.15
CMV	121/124 (97.6)	30/30 (100)	91/94 (96.8)	77/91 (84.6)	>0.43	<0.001
EBV	50/50 (100)	11/11 (100)	39/39 (100)	16/21 (76.2)	1	<0.005

†Serological status of various infectious diseases was examined by the methods described in Patients and Methods. USI, unprotected sexual intercourse; LANA, latency-associated nuclear antigen; CBP, contaminated blood products; TPHA, *Treponema pallidun* hemagglutination test; HBV, hepatitis B virus; HBsAg, anti-hepatitis B virus surface antigen; HBsAb, anti-hepatitis B virus surface antibodies; HCV, hepatitis C virus; HTLV-I, human T-cell leukemia virus type I; HSV-I, herpes simplex virus type 1; VZV, varicella-zoster virus; CMV, cytomegalovirus; EBV, Epstein-Barr virus; ND, not determined.
*Compared by using either χ^2 test or Fisher's exact test.

bodies; herpes simplex virus type 1 (HSV-1), IFA or EIA on anti-HSV-1 IgG; varicella-zoster virus (VZV), IFA or EIA on anti-VZV IgG; cytomegalovirus (CMV), IFA or EIA on anti-CMV IgG; EBV, IFA or EIA on anti-EBV IgG.

RESULTS

There were 32 anti-LANA$^+$ cases in the HIV-1$^+$ cohort: All were males (Table I). Five anti-LANA$^+$ cases were foreigners: two heterosexual Japanese-Latin-Americans, two heterosexual Africans, and one homosexual Caucasian. USI was a risk factor for all 27 Japanese anti-LANA$^+$ cases. Twenty-three patients reported having sex with men. The age distribution of the HIV-1$^+$ cohort ranged from 11 to 68, with a mean of 32.6 ± 9.7 (mean ± 2 standard deviations; S.D.). The mean age of the anti-LANA$^+$ subgroup was 43.8 ± 9.6, whereas that of the anti-LANA$^-$ subgroup was 35.3 ± 8.7, a statistically significant difference ($P < 0.005$). Mean CD4 cell counts were 203 ± 167 in the anti-LANA$^+$ subgroup and 254 ± 204 in the anti-LANA$^-$ subgroup, a statistically insignificant difference. Ten patients with KS were represented in this cohort; and all but one were anti-LANA$^+$.

USI was a risk factor in all 32 patients who were positive for both anti-LANA antibodies and HIV-1. To our knowledge, incidences of various infectious diseases among the HIV-1$^+$ population in Japan have not yet appeared in international journals. Therefore, we compared these incidences according to the major risk factors of HIV-1 infection [USI and contaminated blood products (CBP)] in addition to LANA status (Table II).

The prevalence of syphilis, toxoplasma, HSV-1, CMV, and EBV were significantly higher in the USI subgroup (group I) than in the CBP subgroup (group II). The reverse was true in the cases of HBV (HBsAb) and HCV. Incidences of HBV (HBsAg), HTLV-I, and VZV were not significantly different between the USI and CBP subgroups. When infectious disease incidences were compared according to anti-LANA antibody status, four correlated significantly: Prevalence of amoeba, toxoplasma, HBV (HBsAb), and HSV-1 were significantly higher in the anti-LANA$^+$ USI subgroup than in the anti-LANA$^-$ USI subgroup.

We found only two anti-LANA$^+$ cases in 1,000 HIV$^-$ voluntary blood donors. In fact, there were another two cases in which the sera reacted with BCBL-1; however, these patients were considered anti-LANA$^-$, because they were also positive against Ramos.

DISCUSSION

The cumulative number of HIV-1/acquired immunodeficiency syndrome (AIDS) cases reported to the Ministry of Health and Welfare of Japan was 5,041 at the end of October, 1997. Among these cases, 1,495 (29.7%) were infected by CBP in the early 1980s, whereas USI was a risk factor for virtually all of the remaining cases. Although our HIV-1$^+$ cohort consisted of a slightly higher ratio of CBP cases (45.0%), it roughly represented the epidemiologic characteristics of HIV-1 infection in Japan.

Previously, we identified HHV8 DNA sequences in two Japanese patients with KS [Tachikawa et al., 1996]. In the current study, we detected antibodies against LANA in the plasmas of nine out of ten patients with KS (90%). Anti-LANA$^+$ incidence in KS patients was similar to published results from California, New York, and the United Kingdom [Gao et al., 1996; Kedes et al., 1996; Simpson et al., 1996]. We detected anti-LANA antibodies in 23 cases without KS. USI was a risk factor in all of the anti-LANA$^+$ cases: The great majority of these cases reported homosexual/bisexual intercourse as their risk factor. Anti-LANA antibody-positive status correlated significantly with the pres-

ence of anti-amoeba antibodies and HBsAb, suggesting more active sexual behavior in the anti-LANA$^+$ subgroup than in the anti-LANA$^-$ USI subgroup. As a surrogate marker for male homosexual activity, the presence of anti-amoeba antibody serologically affirmed patient reports. It is interesting to compare the prevalence of HHV8 with that of HBV and HCV. We did not find any anti-LANA$^+$ in HIV-1-infected hemophiliacs in our cohort, which was in sharp contrast to the high prevalence of HBsAb and anti-HCV antibodies among these cases. Both HBV and HCV are transmissible through blood, whereas HBV is more contagious than HCV through USI. HHV8 transmission was related to USI but not to blood products. Perhaps frank viremia of HHV8 is very rare, although the virus may be transmitted through latently infected B lymphocytes.

We found very few anti-LANA$^+$ cases among our HIV$^-$ voluntary blood donors. Higher seroprevalence was reported against HHV8-lytic antigens than against LANA [Lennette et al., 1996]. Further antibody screening using HHV8-lytic antigens and genetic screening using polymerase chain reaction are necessary to ensure blood safety from the virus.

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